African Journal of Biotechnology Vol. 9 (10), pp. 1412-1415, 8 March, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Application of polymerase chain reaction to differentiate between strains of *Campylobacter jejuni* and *Campylobacter coli*

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Accepted 16 June, 2009

A polymerase chain reaction (PCR) assay was used to identify and differentiate between strains of *Campylobacter jejuni* and *Campylobacter coli*. Nine *Campylobacter* reference strains; *C. jejuni* NCTC 11168, *C. jejuni* NCTC 11322, *C. jejuni* NCTC 11828, *Campylobacter coli* NCTC 12110, *C. coli* NCTC 11437, *C. coli* NCTC 11350, *C. coli* NCTC 11366, *C. coli* NCTC 11438, and *C. coli* UA585were included in the assay. DNA primers derived from the genes VAC and SADC were used. PCR amplification of *C. coli* UA585 DNA with consensus sequence primers VAC1 and VAC2 resulted in a single DNA fragment of ~ 705 bp. The forward primer SADC1 and the reverse primer *SADC2* amplified a ~ 1750 bp product of *C. jejuni* NCTC 11168 and *C. jejuni* NCTC 11828. A band of ~ 705 bp was also amplified from *C.* coli UA585 with the two pairs of primers SADC1 and SADC2. The results showed that oligonucleotides primers of VAC and SADC genes can be useful to identify *C. jejuni* and *C. coli*.

Key words: *Campylobacter jejuni*, *Campylobacter coli,* polymerase chain reaction.

INTRODUCTION

The incidence of *Campylobacter* infection has evolved substantially during the last decade as a cause of human gastroenteritis (Linton, 1997). *Campylobacter* species are motile, Gram-negative, curved or spiral rods in shape, oxidase positive and do not ferment or oxidize carbohydrates. *Campylobacter* spp. can be isolated from both domestic and wild animals because the intestinal tracts of warm-blooded animals are a natural reservoir for *Campylobacter* spp. (Lindmark et al., 2004). The most impor-tant pathogenic bacteria belong to thermotolerant, are *Campylobacter jejuni* and *Campylobacter coil* (Sallam, 2007). *Campylobacter* spp. can tolerate and survive at refrigeration temperature for 49 days but do not grow at this temperature (Curtis et al., 2000; Doyle and Roman, 1981). This feature characterizes *Campylobacter* spp. as the most important element in food borne sickness.*C. jejuni* grows in the range of 25-49^{o}C, very sensitive to sodium chloride and drying conditions, and grow well at pH 5.5 to 8.0. In addition, it is capable of growing in human gut and cause severe diarrhea. It causes diarrhea in cattle and septic abortion in both sheep and cattle. Recently, *C. jejuni* and *C. coli* have been recognized as major etiologic agents in human diarrhea disease and associated with human campylobacteriosis (Friedman et al., 2000).

C. jejuni causes Guillain-Barré syndrome (GBS) which is a disorder of the peripheral nervous system leading to temporary paralysis because of acute neuromuscular, and can lead to death (Nachamkin et al., 1998; Hadden and Gregson, 2001). More than 40% of the patients with GBS syndrome were related to infection by *C. jejuni* (Allos, 1997, Nayak et al., 2003) and there was an 80% infection with campylobacteriosis caused by *C. jejuni*. The symptoms of infection by *Campylobacter* spp. are watery diarrhea which develops into bloody diarrhea due to toxins production from some strains of *Campylobacter*. *C. jejuni* produces and releases some toxins such as cytolethat distending toxin (CDT), Shiga toxin, and hemolysins, cholera-related enterotoxins (Picket, 2000).

Recent studies have reported that chicken meats and by products are the major sources of *C. jejuni*. Approxi-

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Table 1. PCR Primers used for *Campylobacter* spp.

Primer	Sequence (5`-3`)
VAC1	5`GTGTAATCATCACCACATTTGATA3`
VAC2	5`TATAGGTTCTCGTGTAGCAATGCT3`
SADC1	5`GGATCCATCACAGCCTTATCACTCAAGTGTC3`
SADC2	5`GATATGCAAGGAAATATCCTAGGTC3`

mately 60% of retail poultry meats and by-products in Japan were contaminated with *C. jejuni*. Comparable levels of contamination were reported in North America and Europe (Suzuki and Yamamoto, 2009). Poultry meats may be contaminated with *Campylobacter* spp. during slaughtering and processing through microbial ingesta or fecal material (Stanley et al., 1998). A 20-40% of chicken meat and chicken meat by-products were contaminated by *Campylobacter* spp. (Suzuki and Yamamoto, 2009). Consumption of contaminated poultry meat or by-products causes human campylobacter infection (Skirrow and Blaser, 1995; Pearson et al., 2000; CDC, 2005).

Conventional culture method for isolation and identification of *Campylobacter* spp. in food samples usually take several days to complete. Alternative rapid, sensitive, and culture independent methods based on PCR amplification have been reported in several studies (Malorny et al., 2003; Jelenik et al., 2005). Recently, oligoneuclotide primers based on certain genes that are specific for *C. jejuni* and for *C. coli* have been described (Gonzalez et al., 1997; Sallam, 2007). Therefore, the objectives of this study were to extract DNA from *C. jejuni* and *C. coli* strains and to utilize a multiplex PCR assay for detecting these strains using oligonucleotide primers.

MATERIALS AND METHODS

Bacterial strains and culture conditions

The following nine reference bacterial strains were tested with the PCR assays following culture on Mueller-Hinton agar at 37°C in microaerobic atmosphere for 48 h: *C. jejuni* NCTC 11168, *C. jejuni* NCTC 11828, *C. jejuni* NCTC 11322, *C. coli* NCTC 12110, *C. coli* NCTC 11437, *C. coli* NCTC 11350, *C. coli* NCTC 11366, *C. coli* NCTC 11438, and *C. coli* UA585.

Polymerase chain reaction

DNA from reference strains of *C. jejuni* and *C. coli* was extracted according to the method of Pitcher et al., 1989. Primer pairs were used as specific hybridization probes to define DNA fragments of VAC and SADC genes. The function of the vaculating cytotoxin VACA is to induce prominent vaculation and degeneration of cultured eukaryotic cells. SADC gene encodes for protein that is highly specific serine transporter. This protein is necessary for *Campylobacter* to use serine as carbon source. The specific oligonucleotides used in the PCR and their sequences are shown in Table 1.

The reaction mixture consisted of 1 µl of DNA bacterial extract, 2 µl of 10X PCR buffer, 0.4 µl of 10X dNTP mixtures, 0.6 µl each of gene primer mix, 0.1 µl of Taq polymerase, and deionized water to a final volume of 15.3 µl. The reaction mixture was amplified in a 9700 GeneAmp® PCR system (Applied Biosystems, Foster City, CA). The PCR cycle used was: heat denaturation at 94°C for 3 min, 35 cycles with denaturation at 94°C for 45 s, annealing at 50°C for 1 min and extension at 72°C for 2 min, final extension at 72°C for 10 min, and holding at 4°C.

3 µl of PCR amplification products was mixed with 1 µl of orange B dye on nescofilm and injected in gel wells. A 1-kb DNA ladder was used as molecular weight marker to evaluate the size bands. PCR products were analyzed by electrophoresis on agarose gels stained with ethidium bromide. Bands were then photographed under ultraviolet transiluminator and the results were evaluated manually.

RESULTS AND DISCUSSION

The two pairs of primers specific for VAC gene was useful in indentifying *C. coli* UA585 strain. Approximatly 705 bp band was observed using VAC1 and VAC2 sequences. However no specific amplification for other *C. jejuni* and *C. coli* were detected (Figure 1). This could be explained by the divergence among target sequence. Three PCR products were visible for *C. jejuni* 11168, *C. jejuni*11828, and *C. coli* UA585 amplified with SADC1 and SADC2 primers. No signals for *C. jejuni* 11322, *C. coli* NCTC 12110, *C. coli* NCTC 11437, *C. coli* NCTC 11350, *C. coli* NCTC 11366, and *C. coli* NCTC 11438 were obtained using the pair of primers (Figure 2). *Campylobacter jejuni* 11168, and *C. jejuni*11828 strains gave the same band size of approximately 1750 bp while *C. coli* UA585 was given approximately 705 bp with primers designed for *SADC* gene. The identical hybridization pattern observed for *C. jejuni* 11168, and *C. jejuni*11828 strains indicated that the SADC gene has same sequence identity for both strains. The obtained band for *C. coli* UA585 using primers specific for SADC gene mean the gene is present in both *jejuni* and *coli* strains. Different size bands for *C. jejuni* 11168, *C. jejuni* 11828, and *C. coli* UA585 indicate the degree of homology shared by the gene of *jejuni* and *coli* strains. Therefore, primers specific for *SADC* gene could be used to distinguish between *jejuni* and *coli*. Further evaluation of the current assay to distinguish between pathogenic and nonpathogenic *Campylobacter* spp is required. Jauk et al. (2003) used PCR-RFLP with restriction enzyme Rsa for detection of *C. jejuni* and *C. coli*. Three fragments with following lengths: 336 bp, 361 bp, 450 bp were obtained from *C. jejuni* and *C. c*oli. Also, the restriction enzyme EcoRV was used for differentiation of

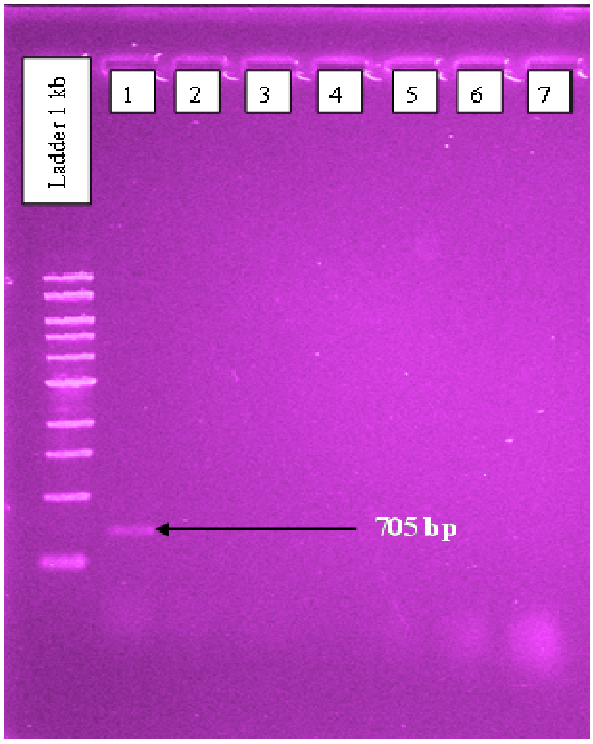

Figure 1. Agarose gel electrophoresis of PCR product obtained with *Campylobacter coli* UA585 specific primer pairs VAC1 and VAC2. The lanes represent the following strains: 1) *C. coli* UA585 2) *C. coli* NCTC 12110, 3) *C. coli* NCTC 11437, 4) *No DNA*, 5) *C. coli* NCTC 11350, 6) *C. coli* NCTC 11366, and 7) *C. coli* NCTC 11438. Lane marked M is DNA size markers (1-kb lader; Biolabs).

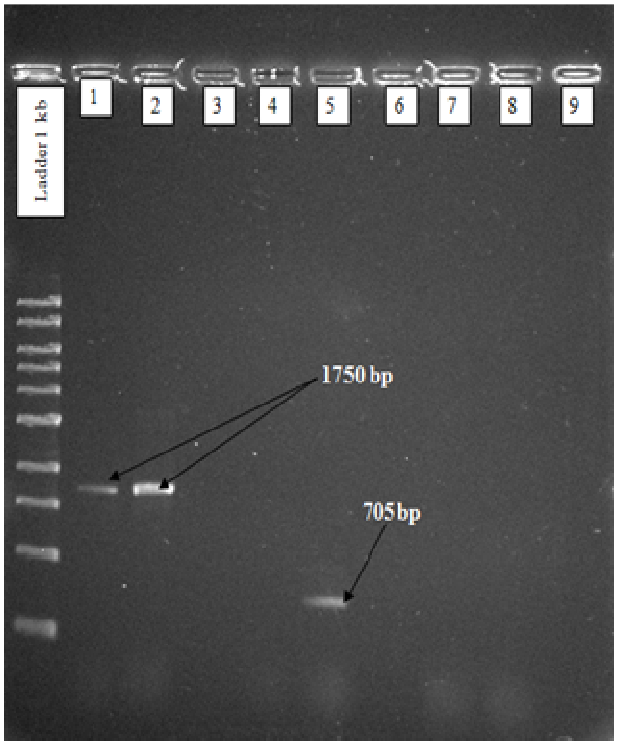

Figure 2. Agarose gel electrophoresis of PCR products obtained with *Campylobacter* 11168, *Campylobacter jejuni* 11828, and *Campylobacter coli* UA585, specific primer pairs *SADC1* and *SADC2*. The lanes represent the following strains: 1) *C. jejuni*11168, 2) *C. jejuni*11828, 3) *C. jejuni* 11322, 4)) No *DNA* 5) *C. coli* UA585 6) *C. coli* NCTC 12110, 7) *C. coli* NCTC 11437, 8) *C. coli* NCTC 11350, and 9) *C. coli* NCTC 11366. Lane marked M is DNA size markers (1-kb lader; Biolabs).

Campylobacter lari from *C. jejuni* and *C. coli*. The banding obtained from *C. lari* was three fragments with following lengths: 290, 346 and 580 bp and it was the same banding obtained from *C. jejuni* and *C. coli.* The PCR system contained two primers located at the start (CF 04) of fla B and at the end of fla A (CF 03). Amplification of the *C. jejuni* and *C. coli* strains yielded two bands of 340 and 380 bp (Steinhauserova and Fojtikov, 1999).

According to Harmon et al. (1997), amplification of the *C. jejuni* ATCC 33560 DNA yielded one band of approximately 460 bp and *C. coli* ATCC 33559 yielded both a 160-bp and a 460-bp fragments. A band of approximately 890 bp was also generated from amplification of DNA from *C. coli* ATCC strain 33559. They used target gene flaA gene and the primer pg3 for *C. coli* and pg50 for *C. jejuni*. Our results showed that oligonucleotides primers of VAC and SADC genes can be useful to identify *C. jejuni* and *C. coli*.

ACKNOWLEDGEMENTS

The author would like to thank the British Council, Riyadh (2006), for funding this project. Thanks are also extended to Mr. Naser Alhumam for his assistance.

REFERENCES

Allos BM (1997). Association between *Campylobacter* infection and Guillain-Barré syndrome. J. Infect Dis. 176: S125-128.

CDC (The US Centers for Disease Control and Prevention) (2005). Campylobacter infection. Available from www.cdc.gov/ncidod/dbmd/diseaseinfo/campylobacter_g.htm.

Curtis LM, Patrick M, Blackburn C, De W (2000). Survival of *Campylobacter jejuni* in foods and comparison with a predictive model. Leatherhead Food Research Association. Research Report, May 1995.

Doyle MP, Roman DJ (1981). Growth and survival of *Campylobacter fetus* subsp. *jejuni* as a function of temperature and pH. J. Food Prot. 44: 596-601.

Friedman CR, Neimann J, Wegener HC, Tauxe RV (2000). Epidemiology of *Campylobacter jejuni* infections in the United State and other industrialized countries. In: Nachamkin I, Blaser MJ (eds): Campylobacter. 2nd ed. ASM Press, Washington, D.C., USA.

Gonzalez I, Grant AK, Richardson TP, Park FS, Collins DM (1997). Specific identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the ceuE gene encoding a putative virulence determinant. J. Clin. Microbiol. 5: 759-763.

Hadden RDM, Gregson NA (2001). Guillain-Barré syndrome and *Campylobacter jejuni* infection. J. Appl. Microbiol. 90:145-154.

Harmon KM, Ransom GM, Wesley IV (1997). Differentiation of *Campylobacter jejuni* and *Campylobacter coli* by polymerase chain reaction. Mol. Cell. Probes, 11: 195-200.

Jauk V, Neubauer C, Szölgyényi W, Vasicek L (2003). Phenotypic and genotypic differentiation of *Campylobacter* spp. isolated from Austrian broiler farms: a comparison. Avian Pathol. 32: 33-37.

Jelenik T, Sabatkova Z, Demnerovam K, Pazlarova J (2005). Two rapid diagnostic procedures for the identification of *Campylobacter jejuni* / *coli* in food matrix. Czech J. Food Sci. 23: 121-125.

Lindmark H, Harbom B, Thebo L, Andersson L, Hedin G, Osterman B, Lindberg T, Andersson Y, Westöö A, Olsson Engvall E (2004). Genetic Characterization and Antibiotic Resistance of *Campylobacter jejuni* isolated from meats, water, humans in Sweden. J. Clin. Microbiol. 42: 700-706.

Linton D (1997). Old and new *Campylobacter*: a review. PHLS Microbiol. Digest, 13: 10-15.

Malorny B, Tassios PT, Radstrom P, Cook N, Wagner M, Hoorfar J (2003). Standardizayion of diagnostic PCR for the detection of foodborne pathogens. Int. J. Food Microbiol. 83:39-48.

Nachamkin I, Allos BM, Ho T (1998). *Campylobacter* species and Guillain-Barré syndrome. Clin. Microbiol Rev. 11: 555-567.

Nayak R, Nawaz M, Khan A, Khan S (2003). *Campylobacter* as a foodborne pathogen and its impact on human health. Recent Res. Dev. Microbiol. 7: 285-606.

Pearson AD, Greenwood MH, Donaldson J, Healing TD (2000). Continuous source outbreak of campylobacteriosis traced to chicken. J. Food Prot. 63: 309-314.

Picket CL (2000). *Campylobacter* toxins and their role in pathogenesis. In: Nachamkin I, Blaser MJ (eds): Campylobacter 2nd ed. ASM Press, Washington, D.C., USA.

Pitcher DG, Saunders NA, Owen RJ (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. 8: 151-156.

Sallam K (2007). Prevalence of Campylobacter in chicken and chicken by-products retailed in Sapporo area, Hokkaido, Japan. Food Control, 18: 1113-1120.

Skirrow MB, Blaser MJ (1995). *Campylobacter jejuni*. In: Infections of the Gastrointestinal Tract. Blaser MJ, Smith PD, Ravodom HB and Guerrant RL Eds. Raven Press Ltd., New York. Chapter 56, pp. 825-848.

Stanley KN, Wallace JS, Currie JE, Diggle PJ, Jones K (1998). Seasonal variation of thermophilic Campylobacters in lambs at slaughter. J. Appl. Microbiol. 84: 1111-1116.

Suzuki H, Yamamoto S (2009). Campylobacter contamination in retail poultry meats and by-products in Japan. A literature survey. Food Control, 20: 531-537.

Steinhauserova K, Fojtikov A (1999). Serotyping and identification of *Campylobacter jejuni* and *Campylobacter coli* strains of human and animal origin using the PCR method. Acta Vet. BRNO, 68: 149-154.